

Optimizing Ammonium Concentration in the Irrigation Solution of Pot-Grown Blueberry (*Vaccinium corymbosum* L.) Plants for Yield and Quality Optimización de la Concentración de Amonio en la Solución de Riego de Plantas de Arándano (*Vaccinium corymbosum* L.) para Rendimiento y Calidad

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SUMMARY

The aim of this work was to optimize the ammonium concentration in the irrigation solution of pot-grown blueberry plants for fruit yield and quality, under protected conditions. An experiment was developed with blueberry (*Vaccinium corymbosum* L.) plants var. Biloxi, with six treatments of ammonium concentrations in the irrigation solutions: 0.2 mM, 2.0 mM, 4.0 mM, 8.0 mM, 12.0 mM, and 16.0 mM, which were repeated four times, generating 24 experimental units (EU), with four plants each. At 200, 215, and 231 days after treatments application the fruits were harvested to evaluate fruit yield (FY), total fruit yield (TFY), proportional fruit yield for each harvest date (PFY), fruit diameter (FD), and soluble solid content (°Brix). The results showed that TFY increased with ammonium concentration until 4.0 mM and beyond slightly decreased; the pattern displayed by PFY showed that plants irrigated with solutions with ammonium concentrations higher or lower than 8.0 mM and 4.0 mM, respectively, delayed their fruit ripening. The effect of increasing N concentration on FD passed from a slight increase to a null effect and to an evident decrease for the first, second, and third harvest dates, respectively. The fruit °Brix increased with ammonium concentration, but decreased 24.0% and varied only 4.6% for the treatments 2.0 mM and 16.0 mM of ammonium, respectively, from the first to the third harvest date. It was concluded that the optimum ammonium concentration in the irrigation solution of blueberry plants grown in pots under protected conditions was 4.0 mM.

Index words: biloxi, growth, hydroponic, nitrogen, productivity.

RESUMEN

El objetivo de este trabajo fue, optimizar la concentración de amonio en la solución de riego de plantas de arándano para el rendimiento y calidad de fruto. Se desarrolló un experimento con plantas de arándano var. Biloxi, con seis tratamientos de amonio en las soluciones de riego: 0.2 mM, 2.0 mM, 4.0 mM, 8.0 mM, 12.0 mM y 16.0 mM; repetidos cuatro veces generando 24 unidades experimentales (UE), con cuatro plantas cada una. A los 200, 215 y 231 días posteriores a la aplicación de los tratamientos, se cosecharon los frutos para evaluar la producción de frutos (PF), la producción total de frutos (PTF), la producción proporcional de fruto para cada fecha de cosecha (PPF), el diámetro de fruto (DF) y el contenido de sólidos solubles (°Brix). Los resultados mostraron que el PTF aumentó con la concentración de amonio hasta 4.0 mM y disminuyó ligeramente más concentraciones más altas. El patrón mostrado por PTF mostró que, las plantas regadas con soluciones con concentraciones de amonio mayores o menores a 8.0 mM y 4.0 mM, respectivamente, retrasaron la maduración de sus frutos; el efecto del aumento de la concentración de N sobre la



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DF pasó de un ligero aumento a un efecto nulo y a una disminución evidente para la primera, segunda y tercera fechas de cosecha, respectivamente. Los °Brix del fruto, aumentaron con la concentración de amonio, pero disminuyeron 24.0% y variaron solo 4.6% para los tratamientos 2.0 mM y 16.0 mM de amonio respectivamente, de la primera a la tercera fecha de cosecha. Se concluyó que la concentración óptima de amonio en la solución de riego fue de 4.0 mM.

Palabras clave: *biloxy, crecimiento, hidropónico, nitrógeno, productividad.*

INTRODUCTION

Blueberries (*Vaccinium corymbosum* L.) are a delightful fruit with high nutritional value, a great source of antioxidants, vitamins, and fiber. The flavor and the beneficial properties for human health of these fruits stimulate their demand and consequently the increase of the production area (Fang, Nunez, Silva, da Phillips, and Munoz, 2020a; Wu and Guan, 2021). In the last decades, between 2000 and 2021, the surface dedicated to blueberry production has exponentially increased worldwide, passing from 211 325 ha to 1 113 261 ha, and in the same period, the imports of this fruit to the USA, the main consumer, also increased exponentially from 16 584 Mg to 259 210 Mg (FAOSTAT, 2023). This plant species is native to the undergrowth temperate forest of North America and requires very particular climatic and soil conditions to achieve optimal growth, development, and yield (Korcak 1989; Metcalfe, Nault, and Hawkins, 2011; Rosen, Allan y Luby, 1990). The unusual requirements of this crop and the increasing demand for its fruits boost the diversification of production systems with innovative horticultural practices to increase the fruit yield and quality and to counteract suboptimal environmental conditions if necessary (Kingston, Scagel, Bryla, and Strik, 2017; Wu and Guan, 2021).

The traditional blueberries open field production is now complemented with protected cultivation systems in glass or plastic greenhouses and by high tunnels covered with plastic and shade net, generally equipped with drip irrigation and with soilless production systems, in containers with several kinds of substrates (Bañados, 2008; Fang et al., 2020a; Jung et al., 2021; Wu and Guan, 2021). In recent years, the combination of high tunnels, pots with substrate, and drip irrigation have become a popular production system in many countries that allows the growing of blueberries in areas with suboptimal adverse climates and soils. On the one hand, the plastic cover protects the plants from extreme temperatures and high light intensities, as well as from wind, rain, hail and birds; on the other hand, the low pot volume, the substrate and the irrigation solution allow better control of root environment, as compared with cultivation in soil (Retamal-Salgado, Bastías, Wilckens, and Paulino, 2015; Li and Bi 2019; Smrke, Veberic, Hudina, Stamic, and Jakopic, 2021a, Smrke et al., 2021b; Smrke, Veberic, Hudina, and Jakopic, 2022). A significant number of works have been carried out to determine the best tunnel characteristics, the pot size, and the composition of the substrates according to the environmental conditions of the production area (Retamal-Salgado et al., 2015; Kingston et al., 2017; Wang, Wang, and Wang, 2018a, Wang, Wang, Chen, Wang, and Wang, 2018b; Li and Bi, 2019; Fang et al., 2020a; Fang, Williamson, Darnell, Li, and Liu, 2020b; Fang, Nunez, Fisher, and Munoz, 2022; Smrke et al., 2022; Yang et al., 2022). Although the nutrient solution used for plant irrigation is a critical component in soilless production systems, limited research has been conducted to determine the optimal composition specifically for blueberry cultivation (Voogt, Dijk, Douven, and Maas, 2014; Tamir, Afik, Zilkah, Dai, and Bar-Tal, 2021; Muñoz, France, Uribe, and Hirzel, 2022). Moreover, fertilization guidelines developed for soil-based systems may not be directly applicable to container-based cultivation (Fang et al., 2020b). In the particular case of nitrogen (N), field-grown blueberry crops typically require annual application rates ranging from 40 to 220 kg ha⁻¹ (Bryla and Machado, 2011; Bañados, Strik, Bryla y Righetti, 2012; Fang et al., 2020b). In contrast, for soilless systems, recommended N concentrations in the nutrient solution generally fall within the range of 70 to 120 mg L⁻¹ (Retamales and Hancock, 2018).

The role of the nutrient solution is to adjust pH and electrical conductivity of the root environment, but mainly to provide water and the mineral essential elements at the appropriate concentrations for plant growth and development (Gorbe and Calatayud 2010; Trejo-Téllez and Gómez, 2012; Resh, 2022). Among these elements, N plays a major role considering that it is the most absorbed element and represents around 3% of plants' dry matter, depending on the plant species, its phenological stage, and the organ. This element is indispensable for the synthesis of amino acids, proteins, and enzymes, which are essential in fundamental physiological processes for plants (Hopkins, 1999; Marschner, 2012). Plants' roots uptake mineral N mainly as nitrate (NO₃⁻) or (NH₄⁺), but most plants prefer the nitric form, even if the energetic costs of its uptake and assimilation into amino acids are higher

(Cárdenas-Navarro, López, Lobit, Ruiz, and Castellanos 2006; Lobit, López, Cárdenas, Castellanos, and Ruiz, 2007; Marschner, 2012; Doyle, Nambeesan, and Malladi, 2021). Indeed, it is usually recommended to provide more than 75% of N as NO_3^- in the nutrient solution used in plant growing soilless systems (Resh, 2022). Nevertheless, it's well established that blueberry plants prefer to absorb N in the ammoniacal form (Merhaut and Darnell, 1995; Claussen and Lenz, 1999; Doyle et al., 2021). Such a preference is associated with the evolutionary adaptation of this plant species to the soil conditions from its original habitat, with low temperatures, high organic matter content, acidic pH, low mineral N availability, and predominance of NH_4^+ (Eck, 1977; Korcak, 1989; Bañados et al., 2012). Moreover, blueberry roots and leaves have a very low content of the enzyme nitrate reductase (NR) and nitrite reductase (NiR), both indispensable in the NO_3^- assimilation process (Claussen and Lenz, 1999; Poornachit and Darnell, 2004; Osorio, Cáceres, and Covarrubias, 2020; Doyle et al., 2021; Leal-Ayala et al., 2021).

In blueberry plants grown in soilless conditions it has been shown that N uptake, tissue N accumulation, leaf photosynthesis, chlorophyll content, plant growth and fruit production are enhanced when the nutrient solution provides N partially or entirely as ammonium (Merhaut and Darnell, 1995; Claussen and Lenz, 1999; Osorio et al., 2020; Xu et al., 2021; Yuan-Yuan, Jun, Jun, Yu-Hui, and Yu, 2021). Nonetheless, though the preference of *V. corymbosum* L. for NH_4^+ is well established, there is a lack of scientific information about the optimal N concentration, supplied exclusively as N-NH_4^+ , in the nutrient solution, for its culture in soilless systems. In such a context, the aim of this work was to optimize the N-NH_4^+ concentration in the irrigation solution of pot-grown blueberry plants, for the analysis of fruit yield and quality.

MATERIALS AND METHODS

An experiment was developed in a tunnel-type greenhouse (9 m L × 12 m W) covered with plastic (30% shading, 7 mm), located at the Instituto de Investigaciones Agropecuarias y Forestales (IIAF), Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), in Morelia, Michoacán, México (19° 46' 11.3" N, 101° 09' 00.1" O; 1860 meters of altitude). The experiment was made with one year old and 0.4 m high 96 blueberry plants (*V. corymbosum* L.) var. "Biloxi", produced in vitro culture and hardened in a greenhouse. During the experiment, inside the greenhouse, the daily average of temperature and relative humidity were 25 °C and 49%, respectively. The plants were placed inside the greenhouse and irrigated once a day with demineralized water during 10 days, then transferred with root ball to 10 L plastic pots, containing a substrate prepared with a mixture of volcanic gravel "tezontle" (0.005 - 0.008 m diameter) and river sand at 1:2 (V/V) ratio, which was previously disinfected with a 10% sodium hypochlorite solution. The plants were homogenized as follows: five days after the transfer, they were thinned to leave a single stem per plant; seven days after, the stem apical part was cut, and seven days later, only one shoot per plant was left, which was supported with plastic stakes during its growth. During the first 30 days after the transfer to the pots, plants received 0.3 L of demineralized water every 12 h (at 9:00 a.m. and 9:00 p.m.), using an automated drip irrigation system. After this period, nutritional treatments were applied, and the demineralized water was replaced by the nutrient solutions.

In the experiment, six treatments were evaluated, each treatment consisted of one N-NH_4^+ concentration in the irrigation solution: 0.2 mM, 2.0 mM, 4.0 mM, 8.0 mM, 12.0 mM, and 16.0 mM. Each treatment was repeated four times, generating 24 experimental units (EU), with four plants each. Six irrigation solutions (one per treatment) were made based on the nutrient solution proposed by Cárdenas-Navarro et al. (1998). All solutions were prepared with demineralized water and had a pH of 5.0, an electrical conductivity of 2.8 dS m⁻¹, H_2PO_4^- 1.0 Eq m⁻³, SO_4^{2-} 28.0 Eq m⁻³, and anions and cations balance of 29.0 Eq m⁻³. The variation of N-NH_4^+ concentrations was compensated by varying the K^+ , Ca^{++} , and Mg^{++} , always keeping the proportion 23, 54, and 23%, respectively. Microelements concentrations were as follows: H_3BO_3 , 42.0 µM; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.0 µM; Fe-EDTA, 15.0 µM; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 23.0 µM; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.3 µM and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 6.0 µM.

From the beginning of the experiment, one plant per EU was randomly selected in order to evaluate the fruit yield (FY); three harvests were made at 200, 215, and 231 days after treatment application (DAT). Total FY (TFY) was determined by the three harvest dates. Proportional FY (PFY) represents the percentage of FY by harvest date from the TFY. The fruits of each EU selected plants were separately weighed (Mettler Toledo, PR8002), their equatorial diameter (FD) was measured with a digital vernier (Truper, 14388), and, in a representative sample, the soluble solids content (°Brix) was determined with a digital refractometer (HI, 96801). TFY was submitted to an analysis of variance (ANOVA) and Tukey mean test ($p \leq 0.05$) when significant statistical differences were found; the mean values of this variable were also adjusted to the following biphasic linear model.

$$TFY = \left(N \leq \frac{a_2 - a_1}{b_1 - b_2} \right) * a_1 + b_1 * N + \left(N > \frac{a_2 - a_1}{b_1 - b_2} \right) * a_2 + b_2 * N \quad (1)$$

Where: TFY = estimated TFY; N = concentration of $N-NH_4^+$ in the irrigation solution; a_1 = y-intercept of the line in the first phase; b_1 = slope of the line in the first phase; a_2 = y-intercept of the line in the second phase; b_2 = slope of the line in the second phase; and $((a_2 - a_1)/(b_1 - b_2))$ = intersection point of the lines. PFY data were transformed with the arcsin function and later submitted to ANOVA, and when significant statistical differences were found, the Tukey mean test ($p \leq 0.05$) was applied. FD and °Brix were adjusted to linear models by harvest date without considering the 0.2 mM treatment because not enough fruits were obtained. All the statistical analyses were carried out with the software IBM® SPSS® Statistics v28(190) for Macintosh (IBM SPSS Statistics, 2021).

RESULTS AND DISCUSSION

The $N-NH_4^+$ concentration in the irrigation solution generated statistically significant differences on TFY (ANOVA, $p < 0.001$); this variable increased significantly when $N-NH_4^+$ was increased from 0.2 mM to 4.0 mM, although non-statistically significant differences were observed when $N-NH_4^+$ was higher (Tukey test $p \leq 0.05$). The relation between TFY and $N-NH_4^+$ concentration was adjusted by a biphasic model. The model predicts that TFY will increase with $N-NH_4^+$ concentration until a critical point, beyond which TFY shows a slight decrease. The critical $N-NH_4^+$ concentration was estimated in 3.98 (≈ 4.0) mM with a maximum TFY of 63.68 g plant⁻¹ (Figure 1; Table 1).

The $N-NH_4^+$ concentration in the irrigation solution likewise caused a statistically significant differences on PFY along the harvest period; blueberry plants irrigated with solutions containing 4.0 mM and 8.0 mM $N-NH_4^+$ produced more than 70% of the TFY in the first harvest date, while the fruit production of plants that received solutions with $N-NH_4^+$ concentrations higher (12.0 mM and 16.0 mM) or lower (0.2 mM and 2.0 mM) produced less than 57% of the TFY; the remaining fruit production was delayed for the second and the third harvest dates (Figure 2A, B and C).

The $N-NH_4^+$ concentration in the irrigation solution also affected the FD during the production period; these effects were fitted with linear models. At the first harvest date FD increased slightly with the increased of $N-NH_4^+$, showing a slightly, but statistically significant slope; in the second harvest date this variable was not affected by the $N-NH_4^+$ treatments, as the slope was non statistically significant and in the third harvest date FD was negatively affected by the increase of $N-NH_4^+$ availability in the irrigation solution, resulting in a statistically significant negative slope (Figure 3 A, B, C and Table 2).

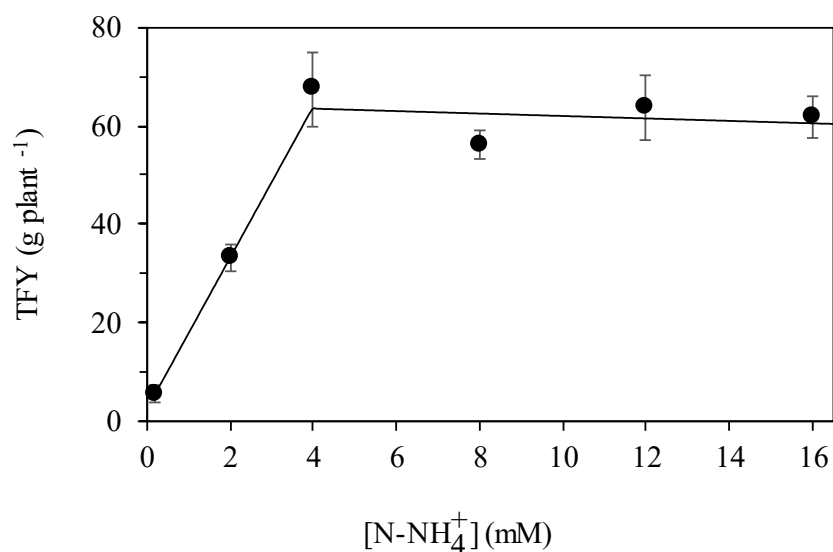


Figure 1. Effect of $N-NH_4^+$ concentration in the irrigation solution on total fruit yield (TFY) of pot-grown blueberry plants. Each point represents the mean of four replicates; error bars show \pm standard error. The solid line shows the fitted linear biphasic model according to the equation and parameters presented in Table 1.

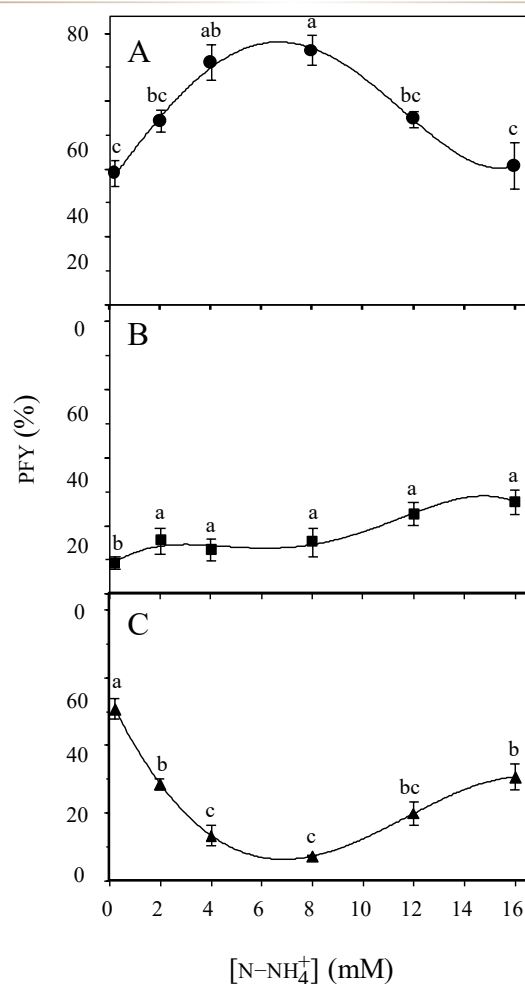


Figure 2. Effect of N- NH₄⁺ concentration in irrigation solution on proportional fruit yield (PFY) of total fruit yield (TFY) of pot-grown blueberry plants for the first (A), second (B), and third (C) harvest. Each point represents the mean of four replicates; error bars show \pm standard error. Different letters in the bars indicate statistically significant differences according to the Tukey test at $p \leq 0.05$. Solid lines show the trend of the mean values.

The °Brix of blueberry fruits increased with the N-NH₄⁺ concentration at the three harvest dates and fit well with linear relationships with statistically significant parameters. However, the fruits of plants that received 2.0 mM N-NH₄⁺ decreased from 13.2 to 10.0 between the first and the third harvest dates, while those of plants that received 16.0 mM N-NH₄⁺ only varied between 14.8 to 14.2 °Brix. Consequently, the value of the parameters *y-intercept* (a) and slope (b) decreased and increased, respectively, from the first to the third harvest dates (Figure 4A, B, C, and Table 3).

Table 1. Linear biphasic model adjusted to the mean values of the total fruit yield (TFY) of pot-grown blueberry plants irrigated with nutrient solutions with several N-NH₄⁺ concentrations.

Adjusted model	R ²	Parameter	Value
$TFY = \left(N \leq \frac{a_2 - a_1}{b_1 - b_2} \right) * a_1 + b_1 * N + \left(N > \frac{a_2 - a_1}{b_1 - b_2} \right) * a_2 + b_2 * N$	0.978	a1	2.451
		b1	15.388
		a2	64.669
		b2	-0.247

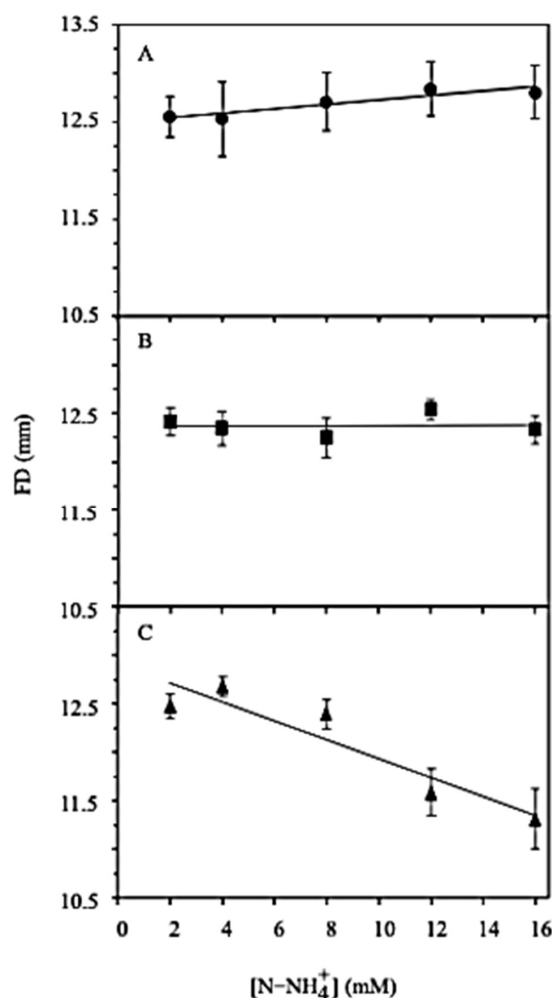


Figure 3. Effect of N- NH_4^+ concentration in irrigation solution on fruit diameter (FD) of pot-grown blueberry plants, at the first (A), second (B), and third (C) harvest dates. Each point represents the mean of four replicates; error bars show \pm standard error. Different letters in bars indicate statistically significant differences according to the Tukey test at $p \leq 0.05$. Solid lines show the linear models fitted for each harvest date, according to the equation and parameters presented in Table 2.

The combination of high tunnels with hydroponics has become one of the most popular blueberry production systems in recent years. Such an intensive system allows to increase the earliness of production, yield, fruit quality, and allows blueberries to be grown in areas with sub-optimal climatic and soil conditions (Bañados *et al.*, 2012; Retamal-Salgado *et al.*, 2015; Li and Bi, 2019; Fang *et al.*, 2020a). In this system, the plants grow in containers with substrates and depend on the nutrient solution, supplied through irrigation, to obtain essential mineral nutrients for their growth and development (Voogt *et al.*, 2014; Kingston *et al.*, 2017; Kingston, Scagel, Bryla, and Strik, 2020; Li and Bi, 2019). The composition of the irrigation solution then becomes critical to the success of the system (Voogt *et al.*, 2014; Fang *et al.*, 2020a; Muñoz *et al.*, 2022). N is considered a main nutrient for plants, due to its essential role in fundamental physiological processes. However, despite its importance for growth, development, fruit production, and quality (Hopkins 1999; Marschner, 2012), very few research works have been carried out to determine the best concentration of this element in the nutrient solution for blueberry plants grown in this production system (Voogt *et al.*, 2014; Muñoz *et al.*, 2022). In a recent work, in one year old soil-grown southern highbush blueberry (SHB *V. corymbosum* L. interspecific hybrid) plants, the optimum N rate for fruit yield was determinate at 206 and 222 Kg N ha⁻¹ year⁻¹ for "Emerald" and "Farthing" cultivars, which correspond to 0.045 and 0.048 Kg N plant⁻¹ year⁻¹, respectively, considering 4600 plants ha⁻¹ (Fang *et al.*, 2020b).

Table 2. Linear models adjusted to the mean values of the fruit diameter (FD) of pot-grown blueberry plants irrigated with nutrient solutions with several N-NH₄⁺ concentrations, at the first (A), second (B), and third (C) harvest dates.

Adjusted model	Harvest date	R ²	Parameters	Value	P
FD = a + b * [N-NH ₄ ⁺]	1st	0.846	a	12.493	<0.001
			b	0.023	0.027
	2nd	0.004	a	12.365	<0.001
			b	0.001	0.919
	3th	0.877	a	12.915	<0.001
			b	-0.098	0.019

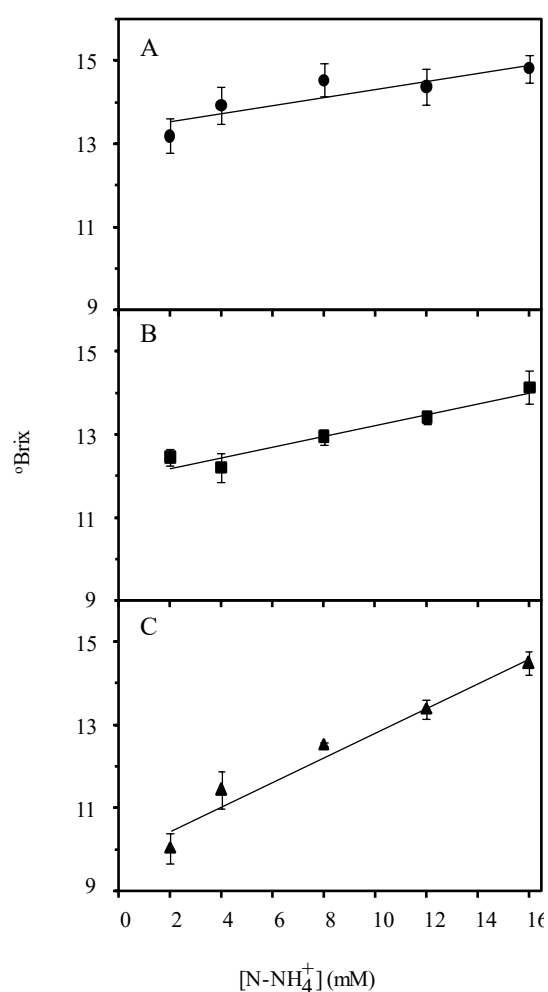
**Figure 4. Effect of N-NH₄⁺ concentration in irrigation solution on degrees Brix (°Brix) of pot-grown blueberry plants at the first (A), second (B), and third (C) harvest dates.** Each point represents the mean of four replicates; error bars show \pm standard error. Different letters in bars indicate statistically significant differences according to the Tukey test at $p \leq 0.05$. Solid lines show the linear models fitted for each harvest date, according to the equation and parameters presented in Table 3.

Table 3. Linear models adjusted to the mean values of the degree Brix (°Brix) of pot-grown blueberry plants irrigated with nutrient solutions with several N-NH₄⁺ concentrations, at the first (A), second (B), and third (C) harvest dates.

Adjusted model	Harvest date	R ²	Parameters	Value	P
°Brix = a + b * [N-NH ₄ ⁺]	1st	0.779	a	13.358	<0.001
			b	0.098	0.047
	2nd	0.937	a	11.939	<0.001
			b	0.131	0.007
	3th	0.964	a	9.891	<0.001
			b	0.296	0.003

Although such data may be a reference, its usefulness is limited in pot-grown plants under high tunnel conditions. In the present study, the response of TFY to increasing concentrations of N-NH₄⁺ in the irrigation solution (ranging from 0.2 to 16.0 mM) followed a classical dose-response pattern, as commonly described in agronomic studies. TFY increased significantly with rising N-NH₄⁺ concentrations up to a critical threshold, beyond which further increases in N-NH₄⁺ supply did not improve yield and instead led to a slight decline (Bray, 1944; Anderson and Nelson, 1975). Based on a biphasic linear model fitted to the experimental data (R² = 0.978; Figure 1; Table 1), the estimated critical threshold for N-NH₄⁺ concentration was 3.98 mM (≈ 4.0 mM). Beyond this point, TFY decreased slightly, most likely due to ammonium toxicity or salinity-induced stress (Machado, Bryla, and Vargas, 2014). Such a concentration, which corresponds to 0.012 Kg plant⁻¹ year⁻¹, appears to be low as compared with previously cited work (Fang *et al.*, 2020b) and to the current N concentrations of most nutrient solutions used in hydroponics, which may reach more than 20.0 mM of this element (Resh, 2022). However, it is close to 5.5 mM N used in some experimental works (Merhaut and Darnell, 1995; Kingston *et al.*, 2017, 2020) and to 3.3 mM N determined in a nutrient solution designated for this crop, based on data from the literature and from plant tissue analyses (Voogt *et al.*, 2014). Other works report good results on plant growth and fruit yield at higher N rates: 12.2-14.7 mM N supplied as organic liquid fertilizer (Clark and Zheng, 2020); 0.058-0.116 Kg N plant⁻¹ year⁻¹ (Muñoz *et al.*, 2022) and 0.021 to 0.030 Kg N plant⁻¹ year⁻¹ (Wilber and Williamson, 2008).

The N-NH₄⁺ availability in nutrient solutions also affected the time to fruit ripening, which is an important factor to consider in order to remain fresh and achieve a good price in the final market (Retamales and Hancock, 2018). The plants irrigated with solutions containing 4.0 mM and 8.0 mM N-NH₄⁺ showed earlier fruit ripening than those irrigated with solutions with lower or higher N-NH₄⁺ concentrations (Figure 2A, B, and C). In the literature, the available information is controversial; on the one hand, in earlier reports, it has been shown that an increase in N concentration delayed fruit ripening in NHB plants growing in open field in soil var. "Wolcot" (Cummings, 1978) or in pot var. "Earliblue" (Eck and Stretch, 1986) and, on the other hand, in more recent reports, the earlier fruit harvest was linearly correlated with the increase of N rate in SHB plant grown also in open field, in soil in var. "Emerald" and "Farthing" (Fang *et al.*, 2020b) or in a container in var. "Misty" (Wilber and Williamson, 2008). Such results suggest that the effect of N availability in the root environment on the earliness of blueberry fruit ripening depends on several factors, such as genotype, plant age, growth conditions, and internal plant reserves of N and C compounds (Wilber and Williamson, 2008; Fang *et al.*, 2020b).

The most important quality factor for blueberry fresh marketing is the fruit size (≈ diameter or weight) (Retamales and Hancock, 2018; Fang *et al.*, 2020b). The results from this work showed that the effect of N-NH₄⁺ concentration in the irrigation solution on FD varies along the fruit production period. At the first harvest, FD increased slightly with the N-NH₄⁺ concentration; however, this effect disappeared in the second harvest, and in the third harvest, the increase of N-NH₄⁺ concentration produced an important decrease of FD (Figure 3A, B, and C; Table 2). The decrease of blueberry fruit size with the increase of N availability in the root's milieu has been previously reported by several authors and explained by the insufficient carbohydrate supply for the fruit-sink demand (Cummings, 1978; Ehret *et al.*, 2014; Vargas and Bryla, 2015; Fang *et al.*, 2020b).

Another important blueberry fruit quality marker is the °Brix (Retamales and Hancock 2018), which was linearly and positively correlated with the N-NH₄⁺ concentration at the three harvest dates of this work. Interestingly, the equation parameters *a* and *b* decreased and increased, respectively, along the production period, because the

experimental data of °Brix of fruits of plants from the treatment 2.0 mM N-NH₄⁺ decreased 24.0% from first to third harvest date, while those of the plants from the treatment with 16.0 mM N-NH₄⁺ only varied by 4.6%. In the literature, the information concerning this subject is scarce, but some authors claim that supplying N fertilizer increases °Brix of blueberry fruits (Jiao *et al.*, 2017; Wang, Jiao, Yin, and Liu, 2022) while others report that this quality fruit marker does not differ among N rates (Ehret *et al.*, 2014; Davis and Strik, 2022).

According to such results, 4.0 mM N-NH₄⁺ in the irrigation solution is proposed as the optimal concentration for blueberry plants grown in pots under protected conditions. At this N-NH₄⁺ concentration, blueberry plants produced the maximum TFY, showed the highest early fruit ripening (statistically = 8.0 mM), exhibited the largest and most constant FD throughout the production period (\bar{X} = 12.5 and SE = 0.1), and more than 86% of TFY (first and second harvest) presented a °Brix value greater than 12.2.

CONCLUSIONS

Fruit production of blueberry plants grown in pots under a protected cultivation system shows a typical yield response pattern to increasing N availability in the root environment (Lawlor *et al.*, 2001; Marschner, 2012). This pattern was fitted by a biphasic linear model that predicts an increasing yield up to an optimal N-NH₄⁺ concentration in the irrigation solution of 4.0 mM, beyond which it decreases slightly. N-NH₄⁺ concentration in the irrigation solution also affected the fruit ripening earliness; when it was higher or lower than 8.0 mM or 4.0 mM, respectively, fruit harvest was delayed. Moreover, the effect of increasing N-NH₄⁺ availability in the root milieu on FD varies along the production period: at the first harvest it was vaguely raised, at the second it remained steady, and at the third it decreased. The °Brix of fruits was linearly and positively correlated with the N-NH₄⁺ concentration in the nutrient solution along the production period; however, from the first to the third harvest dates, this variable decreased by 24.0% and varied only by 4.6% for the treatments 2.0 mM and 16.0 mM, respectively. Considering the results obtained from this work, it was concluded that the optimum N-NH₄⁺ concentration in the irrigation solution for blueberry plants grown in pots under protected conditions was 4.0 mM.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Data sets used or analysed during the current study are available from the first author upon reasonable request.

CONFLICT OF INTEREST

There is no conflict of interest between authors.

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AUTHORS' CONTRIBUTION

Conceptualization: R.C.N., L.L.P., and D.T.H. Methodology: R.C.N., D.T.H., N.L.B.H., and L.L.P. Research: D.T.H., N.L.B.H., and L.L.P. Acquisition of funds: R.C.N. Writing: preparation of the original draft, R.C.N., D.T.H., and L.L.P. Writing, review and editing: R.C.N., and L.L.P.

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